induction of DUSP-1 gene expression was mediated by EP2/EP4 receptors coupled to both the protein kinase A and p38 MAP kinase pathways. In the dorsal air-pouch mouse model of synovial inflammation, LPS-treatments provoked air pouch edema and significant leukocyte infiltration (predominantly neutrophils, CD11c+ monocytes, CD11c+) after 6–24 h, with concomitant increases in TNF-α, IL-1β, MIP-1α, MIP-8, MMP-9, and MMP-13 levels in the exudates. Pre-treatment (30 min) with 1 μmol/L of PGE2 or PGE2 mimetics like forskolin/rolipram reduced LPS-induced leukocyte infiltration by 42±5% (mean±SD) while TNF-α, MIP-1α, MIP-8, MIP-9, and MMP-13 expression levels fell by 67 to 91% on average. PGE2-dependent suppression of induced leukocyte infiltration and MMP-9/TNF-α expression were abrogated in DUSP-1 null (−/−) mice.

Conclusions: We conclude that PGE2-dependent modulation of molecular and cellular components of the inflammatory/proliferative/catabolic response is mediated, at least in part, by DUSP-1. DUSP-1 may be a promising drug target for modulating MAPK-dependent proliferative responses in arthritis, infectious diseases and cancers.

**MITOCHONDRIAL DYSFUNCTION ACTIVATES CYCLOOXYGENASE-2 EXPRESSION IN CULTURED NORMAL HUMAN SYNOVIOCYTES**


**Purpose:** Prostaglandin E2 (PGE2) plays a profound role in the pathogenic processes of rheumatoid arthritis (RA). Recently, it has been reported that mitochondrial alterations may contribute to the progression of RA. In this study, we have investigated the relationship between the dysfunction of mitochondrial respiratory chain (MRC) and the in vitro expression of COX-2 in cultured normal human synoviocytes.

**Methods:** Normal human synoviocytes were isolated from knee synovium obtained from necropsy from 9 adult cadavers (mean age 43 years). Commonly used inhibitors of the MRC were employed to induce mitochondrial dysfunction. Rotenone (1 and 10 μg/ml), 3-nitropropionic acid (0.5, 2 and 10 mM), Antimycin A (AA: 5, 10 and 20 μg/ml), Sodium azide (2, 10 and 25 mM) and Oligomycin (5, 10 and 25 μg/ml) were employed as inhibitor of the complex I, II, III, IV and V of MRC, respectively. Protein and mRNA COX-2 expression were analyzed by cytometry and real time PCR. PGE2 levels were evaluated by ELISA. As a positive control, COX-2 expression was induced by L-arginine (1 ng/ml).

**Results:** Firstly, only the exposure of synoviocytes to AA and oligomycin significantly increased COX-2 protein expression in a time- and dose dependent manner. The maximal response was observed at 6 h with a concentration of 20 μg/ml AA and 25 μg/ml oligomycin (15.4±3.3 and 28.2±10.8 respectively vs. basal 3.6±0.6, n=6). At the same time, the positive control, 1 ng/ml L-arginine, induced a COX-2 protein expression of 45±12.6. When the percentage of cells that expressed COX-2 mRNA was examined by real time RT-PCR the results obtained at 4 h of stimulation were consistent with those of protein expression (30- and 40-fold increase for 20 μg/ml AA and 25 μg/ml oligomycin, respectively, vs. basal 1). The positive control, 1 ng/ml L-arginine, induced a level of COX-2 mRNA expression of 787-fold increase. When the production of PGE2 at 24 h was assessed similar results were obtained (72±23 and 99±38 for AA and oligomycin, respectively vs. basal 23±1). Secondly, we tested if mitochondrial dysfunction induced by AA or oligomycin could modulate the response induced by L-arginine (1 ng/ml) on COX-2 protein expression. We found that pre-treatment of synoviocytes with either 5 μg/ml AA or 10 μg/ml oligomycin for 30 minutes increased significantly the expression of COX-2 induced by L-arginine (1 ng/ml) at protein levels. The values of COX-2 protein expression were 104.7±38.6 for AA + L-arginine and 96.4±24.4 for oligomycin + L-arginine vs. 45.0±12.6 for L-arginine (n=6, p<0.05).

**Conclusions:** These results showed that the dysfunction of mitochondrial respiratory activity induces an inflammatory response in synoviocytes contributing to the chronic inflammation of synovial tissue in RA and aging joint. These data may prove valuable for a better understanding of the participation of mitochondria in the pathogenesis of RA synovium.

**ELEVATED LEVELS OF INFLAMMATORY MEDIATOR PROSTAGLANDIN E2 (PGE2) IN EX-VIVO CULTURED PERIPHERAL BLOOD LEUKOCYTES (PBL) OF OSTEOARTHRITIS (OA) PATIENTS**


**Purpose:** OA is a degenerative joint disease causing loss of joint function, pain and physical disability. The diarthrodial joint’s diseased tissues (bone, cartilage, synovium) are sites of production of cytokines (e.g., II-1β, TNF-α) and inflammatory mediators (e.g., prostaglandins, nitric oxide). We hypothesize that circulating blood cells, exposed to inflammatory mediators as they perfuse the OA joint, may act as sensors reflecting OA disease activity and/or burden. In the current study we explored whether PBL from OA patients are primed to produce inflammatory mediators compared to PBL from healthy controls.

**Methods:** We recruited 56 patients with knee OA and 8 age-matched healthy controls. QRT-PCR was performed using Applied Biosystems. PGE2 levels were measured by ELISA (Cayman) from stored plasma samples.

**Results:** OA patients produced moderately higher levels of PGE2 than healthy controls in unstimulated plasma at baseline (p=0.081). However, when whole blood from both OA and controls was cultured (24 h) ex vivo (100 and 94 pg/ml respectively), PGE2 in controls did not change, while levels in OA patients increased 300% over baseline (p<0.01). The increased PGE2 production at 24 h ex vivo suggested that OA PBLs may be primed or activated in vivo. We therefore examined the COX-2 expression of PBL COX-2, II-1β and TNF-α. Each of these transcripts was elevated in OA patients (p<0.02) compared to controls. PBL levels of II-1β in OA correlated with TNF-α levels (r=0.43, p=0.003); increased COX-2 expression correlated weakly with TNF-α expression (r=0.213, p<0.0003). When stratifying those inflammatory mediator levels in OA patients by NSAID use, we observed higher levels of both baseline PGE2 (p<0.04) and II-1β (p<0.04) in NSAID users compared with non-users. Finally, we asked whether evidence of PBL activation correlated with radiographic findings. PBL PGE2 production significantly correlated with semi-quantitative subchondral sclerosis scores (r=0.37, p<0.013) and negatively correlated with osteophyte scores (r=−0.268, p<0.05). PGE2 levels trended to correlate with increasing KL scores (p=0.1). Relative expression levels of II-1β but not TNF-α moderately correlated (r=0.263, p<0.05) with WOMAC pain score and not with any other x-ray findings. (p=0.4).

**Conclusions:** OA PBL produce higher levels of PGE2 than do age-matched controls. PGE2 production is associated with increased PBL expression of mRNA for COX-2, II-1β and TNF-α. These data indicate that PBL are activated by exposure to inflammatory stimuli as they circulate through the diseased synovium and bone in patients with OA. We propose that activated PBL can serve as biomarkers for disease activity in OA and predict risk for disease progression. Whether the activation of PBL confers a risk of endothelial injury and/or vascular disease over time merits additional evaluation.

**COMMON GAMMA-CHAIN CYTOKINES IN PATIENTS WITH EARLY AND END-STAGE OSTEOARTHRITIS**

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**Purpose:** Innate immune system activation has been implicated in osteoarthritis (OA) pathogenesis, but much of what we know about the synovial inflammatory response in this prevalent joint disease is derived from studies of end-stage patients. In this study, we sought to better characterize cytokine production in patients with early signs of knee OA, focusing on the common gamma chain cytokines IL-15 and IL-21, important mediators linking the innate and adaptive immune response.

**Methods:** Synovial membrane (SM) and fluid (SF) specimens were collected from patients with degenerative meniscal tears and early cartilage degeneration undergoing arthroscopic procedures (early OA) and patients undergoing total knee replacement for end-stage OA. Quantitative real-time PCR was used to compare expression of SM cytokines and cell lineage-specific markers. SF cytokine and matrix-metalloproteinase (MMP-1 and MMP-3) levels (proteases implicated in cartilage extracellular matrix remodeling) were quantified by ELISA. Transcript and protein levels were compared in early and end-stage specimens, using the